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Three Genes Linked to Malignant Epithelial Growth

Researchers at the Howard Hughes Medical Institute (HHMI) at Harvard Medical School have found that three fruit fly genes with close counterparts in human cells are part of the machinery that governs the orderly arrangement and growth of epithelial cells. In humans, the loss of such order is a hallmark of malignant, invasive tumors of skin, breast, colon and other tissues.

Since the functions of the human counterparts to the fly genes remain unknown, the researchers hypothesize that exploration of the human genes could reveal fundamental insights into how tumors arise in epithelial cells.

In an article in the July 7, 2000, issue of the journal *Science*, HHMI investigator Norbert Perrimon and colleagues David Bilder and Min Li at Harvard Medical School report that the *Drosophila* genes, *Scribble* (*Scrib*), *Lethal giant larvae* (*Lgl*) and *Discs-large* (*Dlg*), appear to be part of a genetic pathway that directs epithelial cells to grow in orderly, single-layered sheets. In humans, epithelial cells are found in a variety of tissues, including those that compose the skin, breast and intestines.

In previous studies, Perrimon and his colleagues discovered that a gene mutation poked holes in the outer covering, or cuticle, of fly embryos. They named the gene *Scribble* because the cuticle looked as if a child had scribbled on it. Upon closer examination, the researchers found that the skin cells that make the cuticle had lost their sheet-like organization and polarity—the term used to describe the overall orientation of a cell. In skin cells, polarity is established during development and determines which surfaces of the cells face outward and which face inward.

"Once we had shown that *Scrib* was required for the surfaces of the embryonic epithelia, we turned to look at its role in other epithelia in the fly," said David Bilder, a postdoctoral fellow in Perrimon's laboratory and first author of the *Science* article. "When we made mutations in follicle cells—a second type of epithelium—we saw defects very similar to those in the embryonic epithelia," he said.

Surprisingly, when the researchers looked at a third type of epithelium, the imaginal disc, they found that not only was the epithelium disrupted but the cells within this tissue had become massively overgrown. "The mutant epithelia become solid, tumor-like masses of overgrown cells," said Bilder.

"The loss of cellular architecture in the imaginal discs-which are normally flat Frisbee-like structures-transformed the discs into cellular-like clumps and caused the cells to overproliferate."

With these hints that *Scrib* plays a key role in epithelial organization, the researchers began their search for other genes with similar function. Perrimon and colleagues found two genes, *Lgl* and *Dlg*, that showed effects on the organization of the embryonic and follicular epithelia of flies, just as *Scrib* did. While "*Lgl* and *Dlg* have been studied for decades because they cause imaginal disc cells to overproliferate," said Bilder, "there is not a good understanding of how losing their function produces the overproliferation that is observed".

Intrigued by the similar roles of the three genes in epithelial architecture, the researchers next sought to determine whether they were components of the same regulatory pathway. They used an approach called genetic interaction to slightly turn down the levels of two of the genes at the same time. If the two genes had nothing to do with one another, the fly wouldn't really be affected. But if the genes were part of the same pathway, the further crippling of that pathway would exacerbate the effect. "It's like in a car, if you have a low battery and a cracked muffler, the car still runs fine because the engine and the muffler aren't part of the same system," Bilder said. "But if you have a low battery and weak spark plugs, the car won't start because there are two malfunctions in the electrical system, the pathway that governs ignition." These tests showed that the three genes act together to make epithelial cells grow normally.

To begin to understand how the proteins produced by the three genes might work together, the researchers explored where in the cell the proteins were concentrated. These studies-which included mutating the genes and observing the effects on protein localization-indicate that the *Scrib* and *Dlg* proteins may act together at the cell membrane to recruit the *Lgl* protein from the cytoplasm. Perrimon and Bilder are currently exploring these mechanisms in more detail.

While the fly genes do have close human relatives, understanding the function of the human genes is likely to be far more complicated than in flies, said Bilder. "Most fly proteins have multiple human homologs, often with functional redundancy," he said. "So, while these genes have very dramatic effects when mutated in flies, such effects might be more subtle in humans."

Bilder noted that the relatively simplicity of the fly genes and their effects may offer useful lessons for application to humans. "*Drosophila* tumors are by no means identical to human tumors," he emphasized. "Yet the fly tumors share a remarkable number of characteristics with human malignant tumors, including overproliferation, loss of cell architecture, failure to respond to signals to differentiate, and the ability to invade other organs." The researchers hope that their studies of the fly will assist scientists and clinicians studying human cancer.